

Figure 1:

Sequences producing significant alignments:	Score E	
	(bits)	Value
gi 1168223 sp P35563 5HT3_RAT 5-hydroxytryptamine 3 recepto...	96	8e-19
gi 13242306 ref NP_077370.1  (NM_024394) 5-Hydroxytryptamin...	96	1e-18
gi 9938026 ref NP_064670.1  (NM_020274) 5-hydroxytryptamine...	94	2e-18
gi 2144046 pir  I58179 5HT3 receptor subunit - rat (fragmen...	94	3e-18
gi 4504543 ref NP_000860.1  (NM_000869) 5-hydroxytryptamine...	93	5e-18
gi 13325275 gb AAH04453.1 AAH04453 (BC004453) Unknown (prot...	93	5e-18
gi 9790622 gb AAB37533.2  (S82612) 5-hydroxytryptamine type...	93	5e-18
gi 1586341 prf  2203408A serotonin 3AS receptor [Homo sapiens]	93	7e-18
gi 11559956 ref NP_071525.1  (NM_022189) 5-hydroxytryptamin...	92	1e-17
gi 5174469 ref NP_006019.1  (NM_006028) 5-hydroxytryptamine...	92	2e-17

## Top Alignment

>gi|1168223|sp|P35563|5HT3\_RAT 5-hydroxytryptamine 3 receptor precursor (5-HT-3)  
 (Serotonin-gated  
 ion channel receptor) (5-HT3R)  
 gi|681916|dbj|BAA08388.1| (D49395) serotonin 5-HT3 receptor [Rattus rattus]  
 Length = 483

Score = 95.9 bits (237), Expect = 8e-19

Identities = 81/335 (24%), Positives = 157/335 (46%), Gaps = 28/335 (8%)

Query: 12 LGFSITLLLVHGQG-----FQGTAAIWPSLFNVN--LSKKVQESIQIPNNGSAPLLVDVR 64  
 L ++L+ G+G Q + P+L ++ L ++ ++ + P LV +  
 Sbjct: 10 LALFLSVLIAQGEGRRRATQAHSTTQFALLRLSDHLLANYKKGVPRVDRWRKPTLVSID 69

Query: 65 VFVSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTSAHPR-HAITLEWESLWTPRLTILE 123  
 V + + NVD +++ + R W D L W +++P +S+W P + I E  
 Sbjct: 70 VIMYAILNVDEKNQVLTTYIWYRQFWTDEFLQWTPEDFDNVTKLSIPTDSIWVPDILINE 129

Query: 124 ALWVDWRDQSPQARVDQDGHVKLNALATETNCNFELLHFPRDHSNCSLSFYALSNTAME 183  
 + V P V G V+ L T C+ ++ +FP D NCSL+F + +T +  
 Sbjct: 130 FVDVGKSPSIPYVYVHHQGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTFTSWLHTIQD 189

Query: 184 LEF-----QAHVVN----EIVSVKREYVVYDLKTQVPPQQLVPCFQVTLRLKN 227  
 + ++ +N E++ V ++ + ++T ++ F V +R +  
 Sbjct: 190 INISLWRTPEEVRSDKSIFINQGEWELLGVFTKQEFSTIETSNSYAEMK--FYVVIRRR- 246

Query: 228 TALKSIALLVPAEALLADVCGGLPLRAIERIGYKVTLLLSYLVLHSSLVQALPSSSS 287  
 L ++LL+P+ L++ D+ G LP + ER+ +K+TLL Y V + LP+++  
 Sbjct: 247 -PLFYAVSLLLPISIFLMVVDIVGFCLPPDSGERVSFKITLLGYSVFLIIVSDTLPATAI 305

Query: 288 CNPLLIYYFTILLLLFLSTIETVLLAGLLARGNL 322  
 PL+ YF + + LL +S ET+ + L+ + +L  
 Sbjct: 306 GTPLIGVYFVVCMAILLVISLAETIFIVQLVHKQDL 340

**Figure 2:**

PSSMs producing significant alignments: Score(bits) Evalue

gnl|CDD|4842 pfam02931, Neur\_chan\_LBD, Neurotransmitter-gated ion-channel 1... 54.5 6e-09

CD-Length = 216 residues, only 64.4% aligned  
Score = 54.5 bits (131), Expect = 6e-09

Query:	52	PNNGSAPLLVDVRVFSNVFNVLDILRYTMSSMLLLRLSWLDTRLAWNTSAHP-RHAITLP	110
Sbjct:	19	VRNGGDPVVVSVGLYLQOIISVDEKNQDLTTNVWLRQQWTDPRLAWNPSDYGGITSLRLP	78
Query:	111	WESLWTPRLTILEALWVDWRDQSPQ----ARVDQDGHVKLNALATETNCNFELHFFPRD	166
Sbjct:	79	SDKIWKPDIFLYNK--ADGIHDITTPNTNVRVYPDGTVLWSPPAIYKSSCPMDLTYFPFD	136
Query:	167	HSNCSLSFYALSNTAMELEFQ	187
Sbjct:	137	QQNCSLKFGSWTYNGDEVDLQ	157

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**Figure 3:****Transmembrane Regions:**

Helix 1 from 230 (out) to 253 (in) : Score = 5.26

Helix 2 from 261 (in) to 278 (out) : Score = 3.31

Helix 3 from 295 (out) to 314 (in) : Score = 4.96

Helix 4 from 368 (in) to 390 (out) : Score = 5.47

4 helices (-) : Score = 24.541

**Masked Sequence: >INPIONCH1**

MALWSLLHLTLFLGFSITLLLVHGGQFGTAAIWPSLFNVNLSKKVQESIQIPNNGSAPLL  
VDVRVFSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNTSAHPRHAITLPWESLWTPRLT  
ILEALWVDWRDQSPQARVDQDGHVKLNALATETNCNFELLHFPRDHSNCSLSFYALSNT  
AMELEFQAHVNEIVSVKREYVVYDLKTQVPPQQLVPCFQVTIRLKNLTALKSI~~IALLVPA~~  
~~EALLLADVCGLLPLRAIERIGYKVTLLLSYLVHSSLVQALPSSSSCNPLLIYYFTILL~~  
~~LLLFLSTIETVLLAGLLARGNLGAKSGPSPAPRGEQREHGNPGPHPAEPPSRGVKGSQRS~~  
WPETADRI~~FFLVYVVGVLCTQFVFAGIWMWAACKSDAAPGEAAPHGRRPRL~~

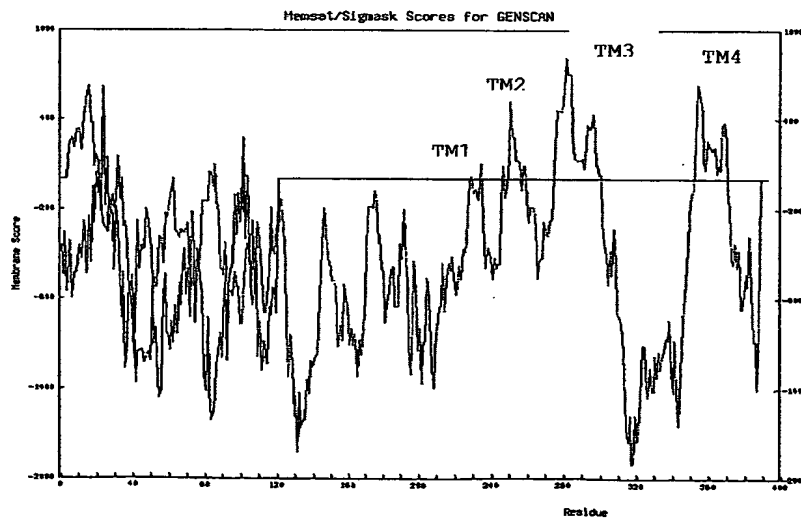


Figure 4:

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5HT3B_MOUSE      -----MILLWSCLLVAVVGILGTATPQ---PGNSSLHRLTRQLLQQYHKEVRPVYNW
5ht3B_RAT         -----MILLWSCLLVAVVGILGTATPQ---PGNSSLHRLTRQLLQQYHKEVRPVYNW
5HT3B_HUMAN       ---MLSSVMAPLWACILVAAG-ÎLATDTHH---PQDSALYHLSKQLLQKYHKEVRPVYNW
5HT3_RAT          MPLCIPQVLLALFLSVLIAQEGESRRRATQAHSTTQPALLRLSDHLLANYKKGVPRVDRW
5HT3_MOUSE        MRLCIPQVLLALFLSMLTAPGEGSRRRATQED-TTQPALLRLSDHLLANYKKGVPRVDRW
5HT3_HUMAN        MLLWVQQALLALLPTLLAQGEARRSRN-----TTRPALLRLSDYLLTNYRKGVRPVDRW
5HT3C_HUMAN       MEGGWPARQSALLCLTVSLLQGRGDAFTINC SGFDQHGVDPAVFQAVFDRKAFRPFNTY
INPIONCH1         --MALWSLLHLTFLGFSITLLLVHGGQGFQG---TAAIWPSLFNVNLSKKVQESIQTPNNG

                                     :: .:

5HT3B_MOUSE      AEATTVYLDLCVHAVLDVDVQNKLTQSVWYREVWNEFLSWNSSLFDEIQEISLPLSAL
5ht3B_RAT         AEATTVYLDLCVHAVLDVDVQNKLTQSMWYREVWNEFLSWNSSLFDDIQEISLPLSAI
5HT3B_HUMAN       TKATTVYLDLDFVHAILDVAENQILKTSVWYQEVWNEFLSWNSSMFDEIREISLPLSAI
5HT3_RAT          RKPTLVSIDVIMYAILNVDEKNQVLTTYIWYRQYWTDEFLQWTFEDFDNVTKLSIPTDSI
5HT3_MOUSE        RKPTTVSIDVIMYAILNVDEKNQVLTTYIWYRQYWTDEFLQWTFEDFDNVTKLSIPTDSI
5HT3_HUMAN        RKPTTVSIDVIVYAILNVDEKNQVLTTYIWYRQYWTDEFLQWNPEDFDNITKLSIPTDSI
5HT3C_HUMAN       SIPTRVNISFTLSAILGVDAQLQLLTSFLWMDLVWDNPPINWNPKCEVGINKLTVLAENL
INPIONCH1         SAPLLVDVRVFNVDVFNVDILRYTMSSMLLLRLSWLDTRLAWNTS-AHPRHAITLFWESL

      . * : . : : : **      : : :      * : : *...      : : . :

5HT3B_MOUSE      WAPDIIINEFVDVERSPLPYVYNSSGTIRNHKPIQVVSACSLQTYAFPPDIQNCSLTF
5ht3B_RAT         WAPDIIINEFVDVERSPLPYVYNSSGTIRNHKPIQVVSACSLQTYAFPPDIQNCSLTF
5HT3B_HUMAN       WAPDIIINEFVDIERYPDLPYVYNSSGTIENYKPIQVVSACSLQTYAFPPDVQNCSLTF
5HT3_RAT          WVPDILINEFVDVGKSPSIPIVYVHHQGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTF
5HT3_MOUSE        WVPDILINEFVDVGKSPNIPIVYVHHRGEVQNYKPLQLVTACSLDIYNFPFDVQNCSLTF
5HT3_HUMAN        WVPDILINEFVDVGKSPNIPIVYIRHQGEVQNYKPLQVVTACSLDIYNFPFDVQNCSLTF
5HT3C_HUMAN       WLPDIFIVESMDVDQTPSGLTAYISSEGRIKYDKPMRVTSICKLDIFYFPFDQONCTFTF
INPIONCH1         WTPRLTILEALWVDWRDQSPQARVDQDGHVKLNALATETNCNFE LLHFPRDHSNCSLSF

      * * : * * : :      . . :      * : .      : : * : :      ** * . * : : :

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5HT3B\_MOUSE NSILHTVEDIDLGLRNREDIEND-KRAFMNDSEWQLLSVSTYHIRQS-SAGDFAQIRF  
5ht3B\_RAT NSILHTVEDIDLGLRNQEDIEND-KRSFLNDSEWQLLSVTSTYHIRQS-SAGDFAQIRF  
5HT3B\_HUMAN KSILHTVEDVDLAFLRSPEDIQHD-KKAFLNDSEWELLSVSTYSILQS-SAGGFAQIQF  
5HT3\_RAT TSWLHTIQDINISLWRTPEEVRSD-KSIFINQGEWELLGVFTKQEFISIETSN SYAEMKF  
5HT3\_MOUSE TSWLHTIQDINITLWRSPEEVRSD-KSIFINQGEWELLEVPQKFEFSIDISN SYAEMKF  
5HT3\_HUMAN TSWLHTIQDINISLWRLPEKVKSD-RSVFMNQGEWELLGVLPYFREFSMESSNYYAEMKF  
5HT3C\_HUMAN SSFLYTVDSMLLGMDKEVWEITDTSRKVIQTQGEWELGIN-KATPKMSMGNNLYDQIMF  
INPIONCH1 YALSNTAMELEFOAHVVN-----EIVSVKREYVVYDLKTQVPPQOL-----VPCFQV  
: \* .: : . . \*: : :

5HT3B\_MOUSE NVVIRRCPLAYVVVSLIPSIIFMLVDLGSFYLPNCRARIVFKTNVLVGYTVFRVNMSDE  
5ht3B\_RAT NVVIRRCPLAYVVVSLIPSIIFMLVDLGSFYLPNCRARIVFKTNVLVGYTVFRVNMSDE  
5HT3B\_HUMAN NVVMRRHPLVYVVVSLIPSIIFMLVDLGSFYLPNCRARIVFKTSVLVGYTVFRVNMSNQ  
5HT3\_RAT YVVIRRRPLFYAVSLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDT  
5HT3\_MOUSE YVIIRRRPLFYAVSLLPSIFLMVVDIVGFCLPPDSGERVSFKITLLLGYSVFLIIVSDT  
5HT3\_HUMAN YVVIRRRPLFYVVSLLPSIFLMVMDIVGFYLPNDSGERVSFKITLLLGYSVFLIIVSDT  
5HT3C\_HUMAN YVAIRRRPSLYIINLLVPSSFLVAIDALSFYLPASENRPFFKITLLLGYNVFLLMNDL  
INPIONCH1 TLRKNTALKSIIALLVPAEALLADVCGGLPLRAIERIGYKVTLLLSYLVHSSLVQA  
: :. . : \*\*: \*: \* . \*\* . \* :\* .:\*. \* \*: :

5HT3B\_MOUSE VPRSAGCTPLIGVFFTVCMALLVLSLSKSILLIKFLYEE-----RHSGQERP--L  
5ht3B\_RAT VPRSAGCTSLIGVFFTVCMALLVLSLSKSILLIKFLYEE-----RHSEQERP--L  
5HT3B\_HUMAN VPRSVGSTPLIGHFFTICMAFLVLSLAKSIVLVKFLHDE-----QRGGQEQP--F  
5HT3\_RAT LPATAIGTPLIGVYFVVCMA LLVISLAETIFIVQLVHKQDLQRPVPDWRHLVLDRIAWL  
5HT3\_MOUSE LPAT-IGTPLIGVYFVVCMA LLVISLAETIFIVRLVHKQDLQRPVPDWRHLVLDRIAWI  
5HT3\_HUMAN LPATAIGTPLIGVYFVVCMA LLVISLAETIFIVRLVHKQDLQQPVPAPWLRLVLERIAWL  
5HT3C\_HUMAN LPAS--GTPLISVYFALCLSLMVVSLETVFITYLLHVATT-----QPPMPRWLHS  
INPIONCH1 LPSSSSCNPLIYYFTILLLLFLSTIETVLLAGLLARG-----  
:\* : ..\*: :\*. : : :\*: : : : :

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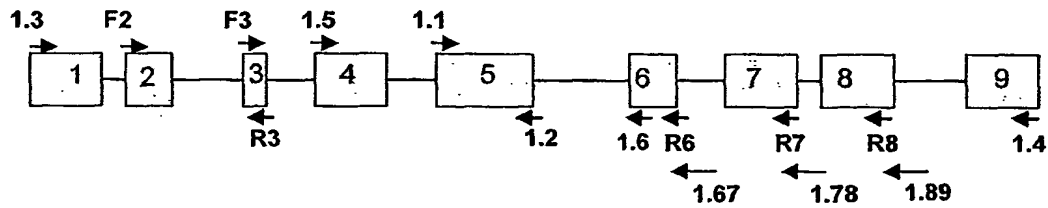
6/19

5HT3B\_MOUSE MCLQGDSDAEESRLYLGA PR-----ADV TES PVHQEHRVPSD  
5ht3B\_RAT MCLRGSDANESRLYLRA PC-----AEDTESPV RQEHQVPSD  
5HT3B\_HUMAN LCLRGDTDADRPRVEPRA QR-----AVVTESSLYGEHLAQPG  
5HT3\_RAT LCLGEQPMahrppatfQANKTDDCS-----AMGNHCSHV GSPQDLEKTSRSRDSPLPPP  
5HT3\_MOUSE LCLGEQPMahrppatfQANKTDDCSGSDLLPAMGNHCSHVGGPQDLEKTPRGRGSPLPPP  
5HT3\_HUMAN LCLREQSTSQRPPATSQATKTDDCS-----AMGNHCSHMGGPQDFEKS PRDRCSPPPPP  
5HT3C\_HUMAN LLLHCTSPGRCCPTAPQKGN-----KGLGLTLTHLP GPKEPG  
INPIONCH1 -NLGAKSGPSPAPRG-----EQREHG NPGPHPAE

5HT3B\_MOUSE -----TLKDFWFQFRSINNSLRTRDQIHQKEVEWLAILYRFDQLLFRIYLA VLGLYTVTL  
5ht3B\_RAT -----TLKDFWFQLQSINNSLRTRDQVYQKEVEWLAILCHFDQLLFRIYLA VLGLYTVTL  
5HT3B\_HUMAN -----TLKEVWSQLQSI SNYLQTQDQDQAEWLVL LSRFDRLLFQSYLFMLGIY TITL  
5HT3\_RAT REASLAVRGLLQELSSIRHSLEKRDEMREVAR DWLRVGYVLDRLLFRIYLLAVLAYSITL  
5HT3\_MOUSE REASLAVRGLLQELSSIRHFLEKRDEMREVAR DWLRVGYVLDRLLFRIYLLAVLAYSITL  
5HT3\_HUMAN REASLAVCGLLQELSSIRQFLEKRDEIREVAR DWLRVGSVLDKLLFHIYLLAVLAYSITL  
5HT3C\_HUMAN -----ELAGKKLGPRETEPDGGS AWTKTQLMELWVQFSHAMD TLLFRLYL LFMASILT V  
INPIONCH1 -----EPSRGVKGSQRSWPETADRIFFLVYVVGVLCTQFV FAGIWMWAACKSDAAPGE

5HT3B\_MOUSE CSLWALWSRM  
5ht3B\_RAT CSLWALWSRM  
5HT3B\_HUMAN CSLWALWGGV  
5HT3\_RAT VTLWSIWHYS  
5HT3\_MOUSE VTLWSIWHYS  
5HT3\_HUMAN VMLWSIWQYA  
5HT3C\_HUMAN IVLWNT----  
INPIONCH1 AAPHGRRPRL

Figure 5:



IC1.1        GCTCTGGGTGGACTGGAGGG

IC1.2        CCGTGTTGCTGAGAGCGTAGAAGC

IC1.3        CCGGAATTCATGGCCCTATGGTCCCTGCT

IC1.4        CCCAAGCTTTTACAGTCTAGGCCGCCTGC

IC1.5        GACACTCGCCTGGCCTGGAACACTA

IC1.78       GCAACCCACTGCTCATTACTACTT

IC1.R3       CAGCCTAAGCAGCAGCATGG

IC1. R7       TGAGCAGTGGGTTGCAGGAG

IC1.F2       TCTGGCCATCCCTCTTCAAC

IC1.F3       GACATCCTGCGATAACAAT

Figure 6:

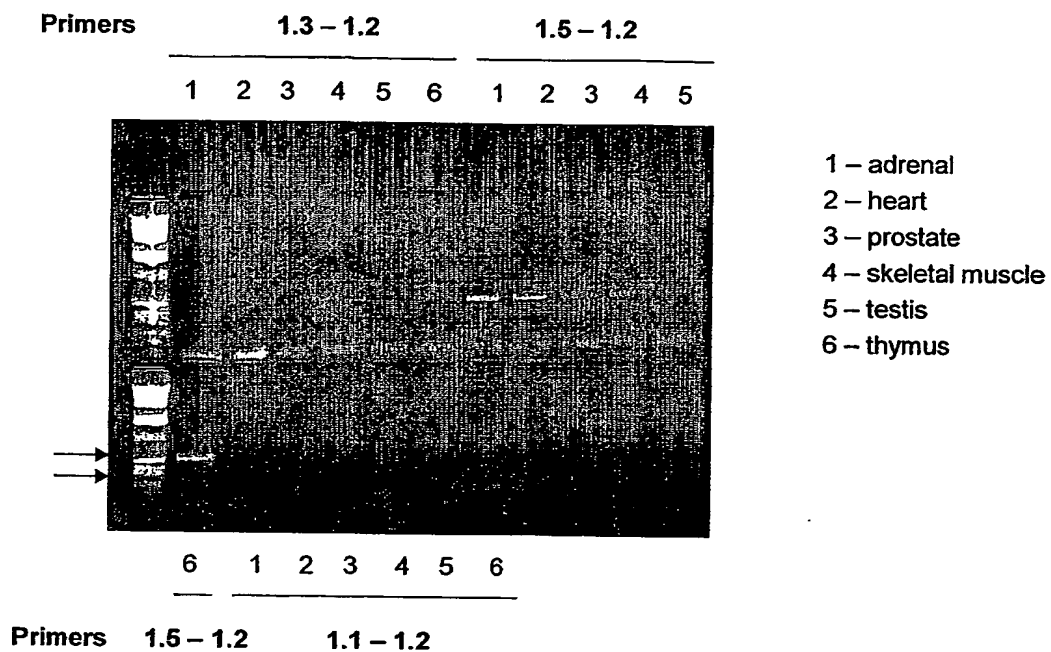




Figure 7:

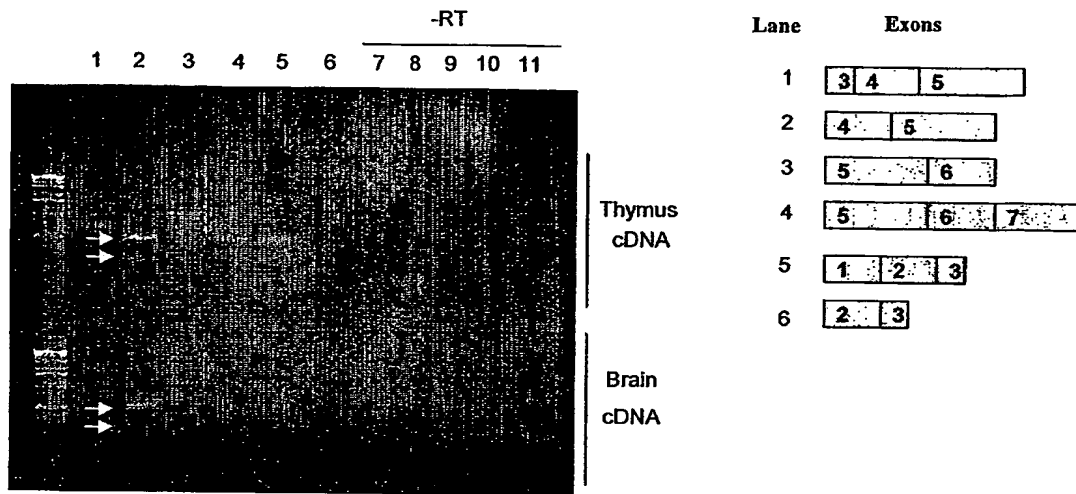


Figure 8A:

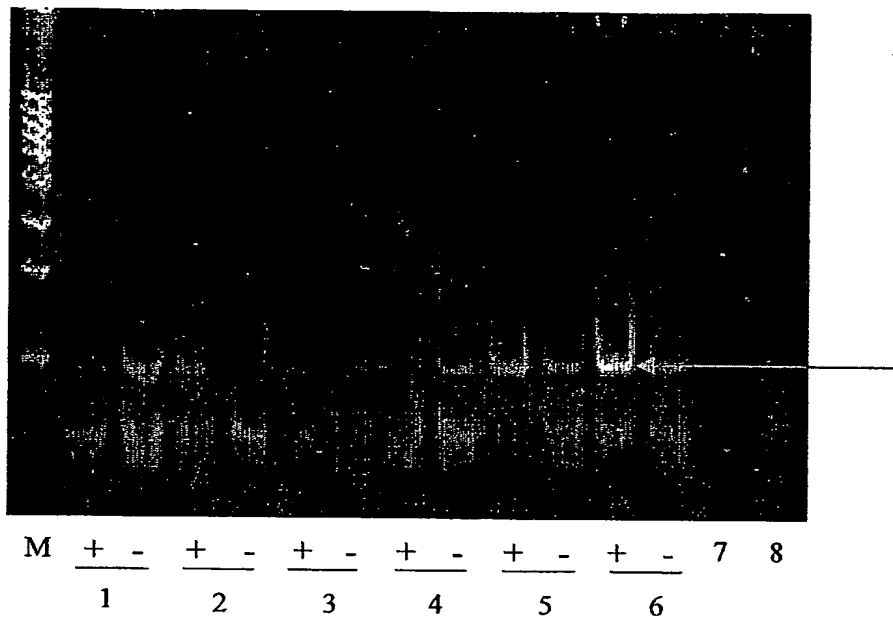


Figure 8B:

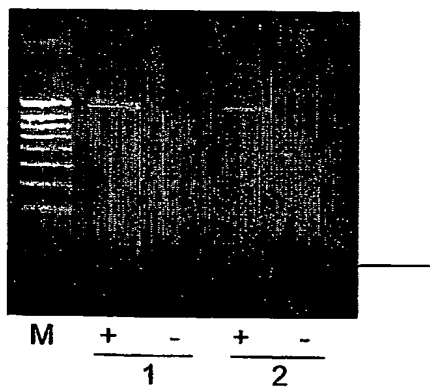
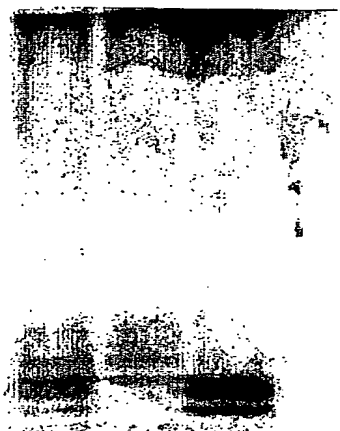


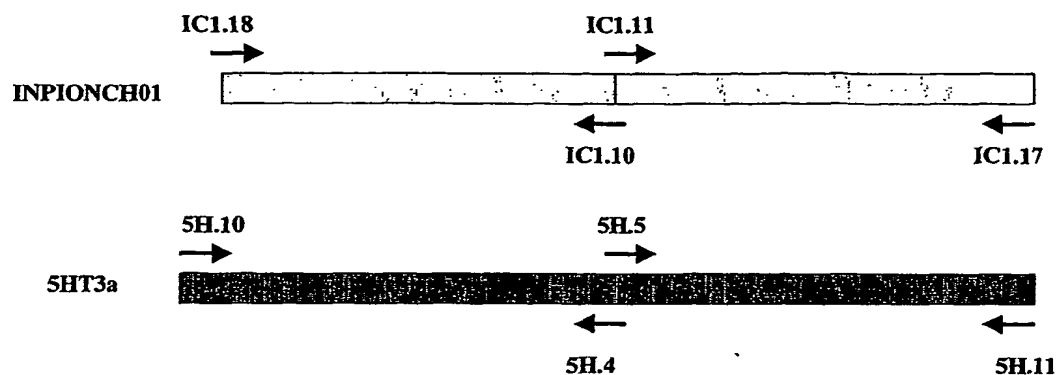
Figure 9:



1 2 3 4

- 1 - 5HT3a + INPIONCH01
- 2 - 5HT3a
- 3 - INPIONCH01
- 4 - untransfected cells

Figure 10:



IC1.10 CCGGAATTCCGCCGTGTTCTTCAGCCTCAG

IC1.11 CCGGAATTCCTCAAGTCCATCATCGCTCTC

IC1.17 CTAGTCTAGACTCAGTCTAGGCCGCCTGCCAT

IC1.18 CCCAAGCTTGCCACCATGGCCCTATGGTCCCTGCT

5H.4 CCGGAATTCGCGGATGACCACATAGAACTTC

5H.5 CCGGAATTCGCGCGGCCCTCTTCTATGTG

5H.10 CCCAAGCTTGCCACCATGCTGCTGTGGGTCCAGCA

5H.11 CTAGTCTAGACTAGCGTACTGCCAGATGGACCAGAGCAT

Figure 11:

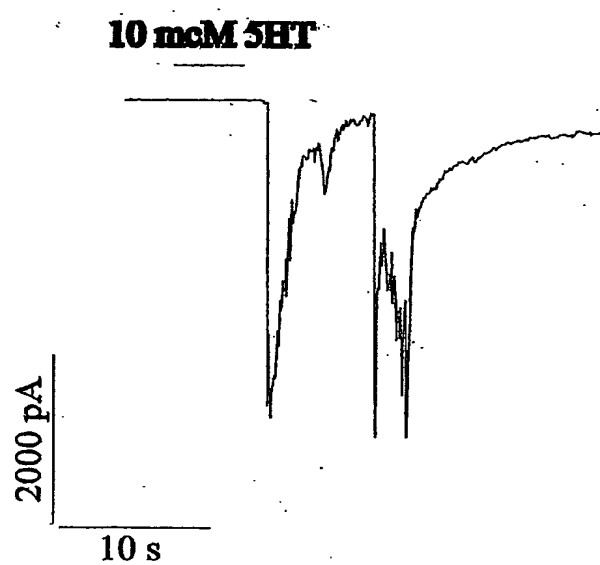


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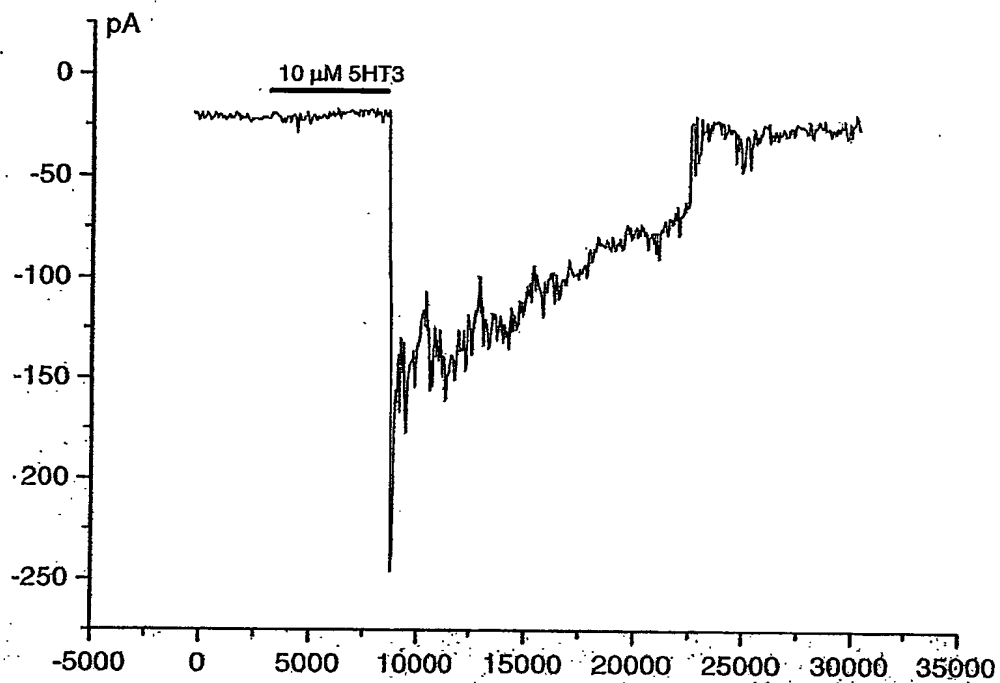


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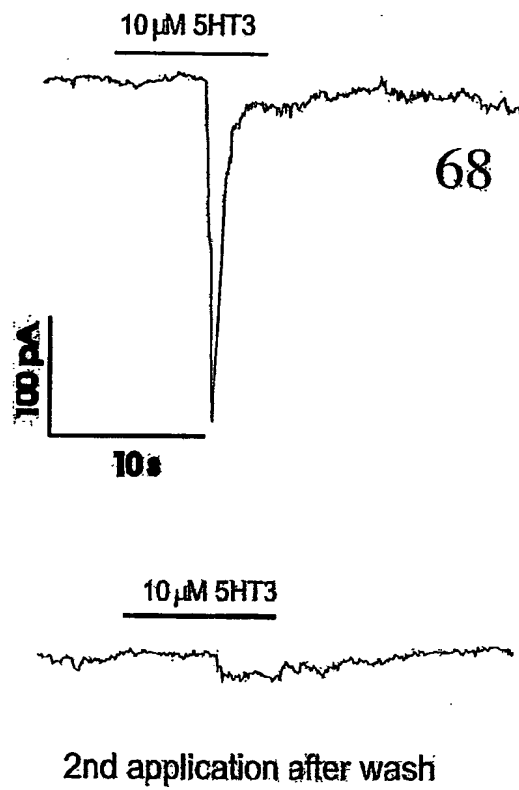


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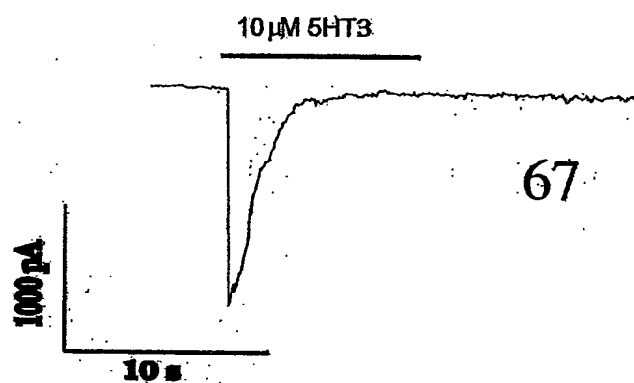
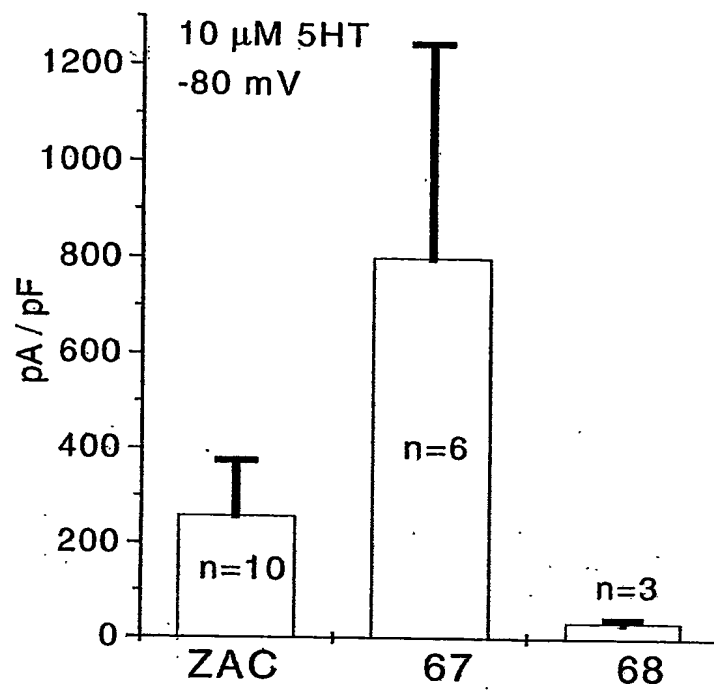




Figure 15:



Amplitude of serotonin-induced  
(10  $\mu$ M) currents.

**FIG. 16**  
HEK293 cells expressing INPIONCHO1  
Current induced by 10  $\mu$ M 5-HT

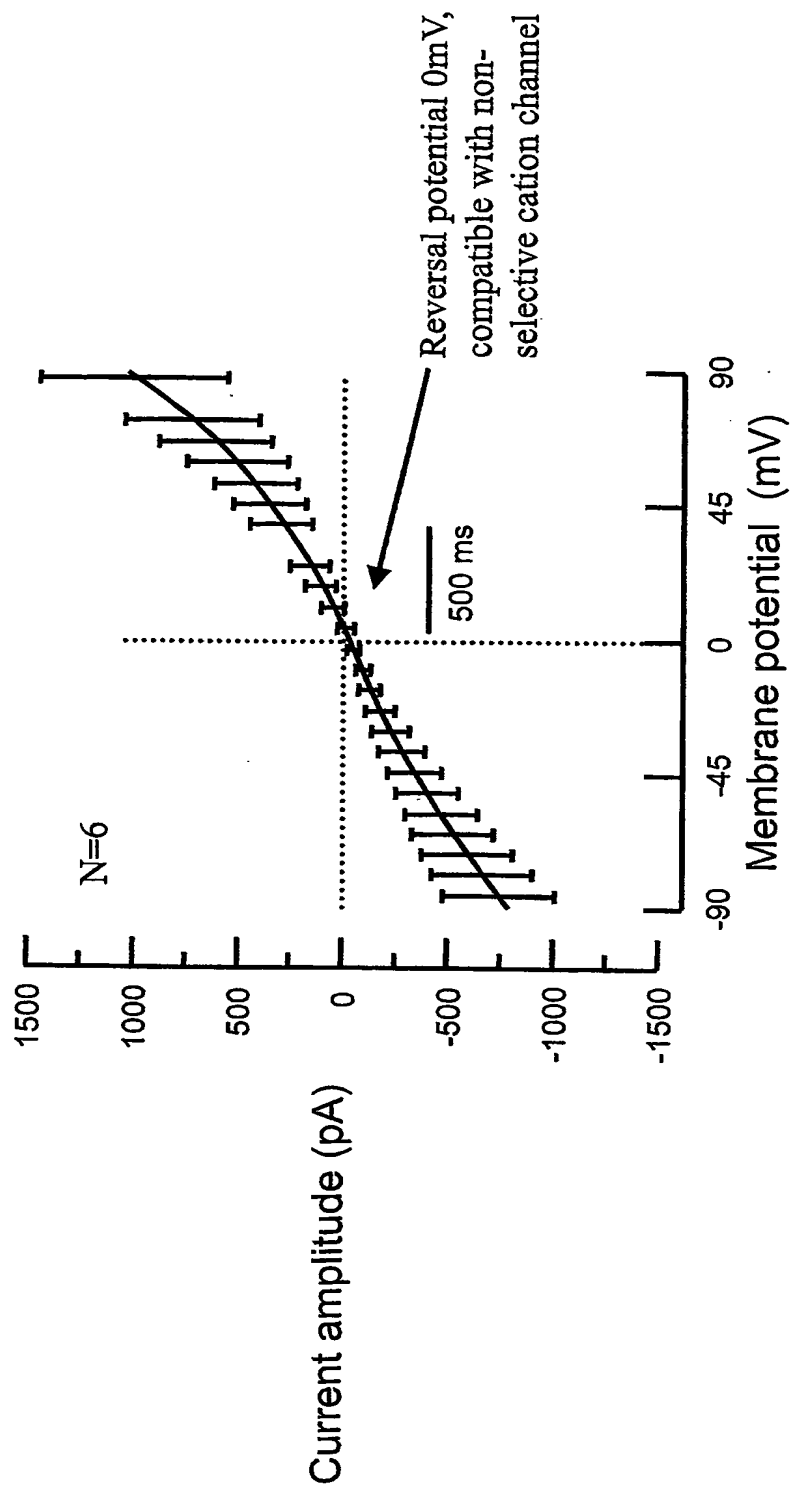


Figure 17:

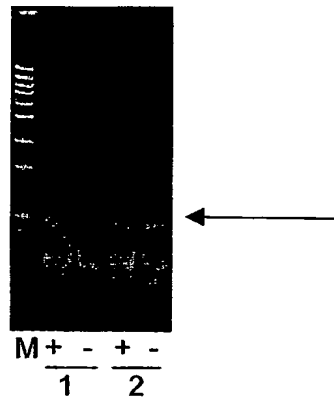
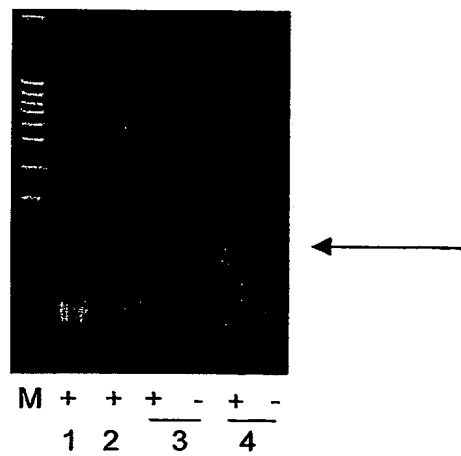


Figure 18:



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